TANK EXPERIMENTS TO QUANTIFY FATE OF MICROCYSTIN IN SHALLOW COASTAL SEDIMENTS

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Abstract

Harmful algal blooms (HABs) have become more prevalent within Lake Erie since the mid-1990s. Microcystin is one of the most common and harmful toxins associated with HABs, yet little is known about its attenuation and fate in the environment. Microcystin is a cyclic heptapeptide with 2 variable L-amino acids, which differentiate between the over 60 variants of microcystin known. Variants have differing toxicity profiles, the most toxic being microcystin-LR, which contains leucine (L) and arginine (R) amino acids. The goal of this thesis research was to determine whether wave-driven benthic exchange accelerates the attenuation of microcystin in shallow coastal waters using laboratory wave tank experiments. Sediment was collected from Western Lake Erie and incorporated into a 110-gallon tank. A solution consisting of both a conservative chloride tracer and microcystin-LR stock dissolved in water was added to surface water at the start of both a Wave Trial and a Non-Wave Trial, and concentrations were monitored over time in surface water and shallow pore water. Results show that wave conditions had a significant impact on exchange rates of conservative chloride, mixing the system over 30 times faster than stagnant conditions. Microcystin concentration in surface water and pore water decreased faster than chloride, likely due to sorption to sediments, degradation, or both. It is crucial to better understand microcystin attenuation and mechanisms responsible in order to accurately predict the severity, duration, and extent of algal toxin plumes, which negatively affect the health of coastal ecosystems and economies.

ACKNOWLEDGEMENTS

First, I would like to thank my research advisor, Dr. Audrey Sawyer, for her enthusiastic guidance, encouragement, and expertise. I would also like to thank her for spending an entire Friday afternoon driving to Lake Erie and back with me to sieve fresh sediment. Without her, this project would not be possible. I would also like to thank my mentor, Kelsey Danner for generously extending her knowledge and experience, and going out of her way to help me in any way she could, including spending hours in the lab helping with sample analyses. I would like to extend my appreciation to Dr. Sue Welch for sharing her extensive geochemical knowledge with me and teaching me how to run samples on the Ion Chromatograph. Further appreciation is extended to my collaborator, Dr. Jiyoung Lee from the College of Public Health, who allowed me to utilize her laboratory facilities to conduct my experiments and shared her expertise on microcystin. I would also like to thank to Dr. Lee's graduate student, Igor Mrdjen, for running the ELISA assays. This research was supported through a Research Scholar Award from The Ohio State University's Undergraduate Research Office, a Grant-In-Aid of Research Award from Sigma Xi, Ohio Sea Grant R/ES-022, and NSF GEO EAR IF 1342632.

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Introduction

Harmful algal blooms (HABs) are increasingly prevalent in lakes and reservoirs and have become a major environmental problem worldwide [*Heisler et al.,* 2008]. HABs pose annoyances to recreationists, reduce water quality for fish, and therefore negatively affect local economies and fisheries. HABs persist in environments with adequate supply of nitrogen and phosphorous, over a wide range of temperatures and pH values, and thrive with eutrophication in warm, sunny climates [*Heisler et al.,* 2008]. Major sources of nitrogen and phosphorous include fertilizer used in agriculture and landscaping, and leaky septic tanks [*Korleski,* 2010]. Unnaturally high nutrient loads are transported from land to lakes via land-surface runoff and groundwater flow. Consequently, the consistent increase in the occurrence of HABs can be attributed to anthropogenic nutrient enrichment, modification of the natural water cycle, and climate change [*Bruno et al.,* 2012].

Microcystis is one particularly harmful algal genus that is often present in HABs. Upon Microcystis cell lysis, the cyanobacteria release a liver toxin known as Microcystin [*NOAA,* 2009]. Over 60 variants of microcystins are known, one of the most common and toxic being microcystin-LR (MC-LR) [*Hyenstrand et al.,* 2001]. MC-LR presents serious hazards to humans through direct consumption, contact during recreational activities, and indirect consumption of contaminated food [*Codd et al.,* 1999]. The World Health Organization has set a drinking water guideline of 1 $\mu g\ L^{-1}$ and a recreational guideline of 20 $\mu g\ L^{-1}$ because higher concentrations have been shown to cause sickness and liver failure in livestock and other terrestrial and aquatic organisms [*Chen et al.,* 2008]. MC-LR is especially concerning as it was detected in 96% of Midwestern United States lakes in a 2009 survey [*Graham et al.,* 2010]. Among these are the Great Lakes, which make up 90% of the United States' freshwater supply [*NOAA,* 2009]. Lake Erie, the site of focus for this study, experienced the largest HAB in recorded history in 2011 [*Michalak et al.,* 2013]. During the event, MC-LR concentrations were estimated to have reached over 4,500 $\mu g\ L^{-1}$ [*Michalak et al.,* 2013]. In August 2014, another microcystin episode near the water intake structure for the city of Toledo led to a tap water ban for over 500,000 people.

New research is improving our understanding of microcystis dynamics in the environment [*Ihle,* 2005]. Yet, despite the increasing concern that MC-LR poses, the mechanisms controlling MC-LR degradation and attenuation in the water column are still poorly understood. Common attenuation mechanisms for organic toxins include photodegradation, sorption to sediments, biological degradation, and dispersion. Previous studies have suggested that photodegradation is minimal, in part because toxins often co-occur with HABs that reduce light penetration in the water column [*Chen et al.*, 2008]. The sorption affinity of MC-LR is also moderate to minimal, depending on the organic and clay content of sediments [*Wu et al.*, 2011]. Biodegradation is therefore likely to be a primary attenuation mechanism, along with dispersion. Laboratory studies have shown that the greatest biodegradation rates occur in the presence of oxygen and sediment-dwelling bacteria [*Song et al.*, 2014]. It is therefore possible that the exchange of surface water through shallow sediments (i.e., benthic exchange) enhances biodegradation of MC-LR by delivering the toxin to shallow, aerobic sediments.

Several mechanisms can drive benthic exchange, but one of the more common mechanisms in lakes is the interaction of waves with the sediment-water interface. A previous study by Precht and Huettel *[2003]* used tank experiments to model the effects of waves on benthic exchange. They found that the interaction of waves with bedforms greatly amplified the rate and depth of

solute exchange across the sediment-water interface relative to molecular diffusion. It is still unclear, however, how benthic exchange influences the persistence of MC-LR in the water column, making it difficult to predict the fate and transport of MC-LR in water bodies such as Lake Erie. Specifically, it is unclear how MC-LR degrades in surface water and shallow sediments and whether dispersal is the main mechanism for attenuation or if other processes such as degradation and sorption play additional roles. The primary purpose of this study is to use an experimental tank to quantify the mobility of MC-LR in shallow lake sediments and surface waters in the presence and absence of wave-driven benthic exchange (wave pumping). Such experiments show that waves increase solute exchange between surface water and pore water, and that MC-LR is rapidly removed from the water column, suggesting that sorption and degradation may play key roles in attenuation.

METHODS

Overview of Experimental Methods

Tank experiments included two trials (with and without waves) to compare the effects of both wave driven benthic exchange and molecular diffusion on MC-LR attenuation. Coastal Lake Erie sediment was sieved to 0.178 mm and combined with medium-grained silica #20 pool filter sand and then added to a 110-gallon glass aquarium tank. Total sediment depth was 8 cm. Sediment was then covered with an additional 12 cm of reverse osmosis (RO) water for each trial (Figure 1). Total dissolved solids (TDS) in RO water were measured to be >0.05 ppt using the Aqua Troll 200. In the trial with waves, a Jebao WP-25 wave maker created a consistent wave height of 2 cm and period of 1 second and resulted in quick development of bedforms on the sediment surface. Bedforms developed before the start of the Wave Trial as the wave maker was run for about 24 hours prior to allow for temperature equilibration and other conditions.

At the start of each trial, a solution consisting of 26 g of chloride salt and 0.77 mg of MC-LR stock dissolved in 1 L of RO water were added to surface water. These specific concentrations were added in order to achieve an initial concentration of approximately 237 mg L^{-1} chloride and 11.5 μg L^{-1} MC-LR in the 67-liter-volume of water overlying the sediment bed. Chloride was added to the liter of water first and allowed a few hours to fully dissolve. The MC-LR stock was added to the same liter of water several minutes prior to addition to the tank. The solution was held in a 1 L glass container with a cap. Both MC-LR stock and a conservative chloride salt tracer were added at the start of each trial to achieve an initial surface water concentration of approximately 237 mg L^{-1} chloride and 11.5 μg L^{-1} MC-LR.

Throughout both trials, samples were collected to test for concentrations in surface water and pore water at 2 cm, 4 cm, and 6 cm depths (Figure 1). Specific conductivity was also monitored in the surface water as a proxy for chloride using an In-Situ Inc. Aqua Troll 200. Temperature, specific conductivity, dissolved oxygen, pH, TDS, and oxidation-reduction potential were also measured at the beginning and end of each trial. More information regarding materials, tank setup, and reagents can be found in the Standard Operating Procedure (Appendix I) along with specifics regarding experimental procedures

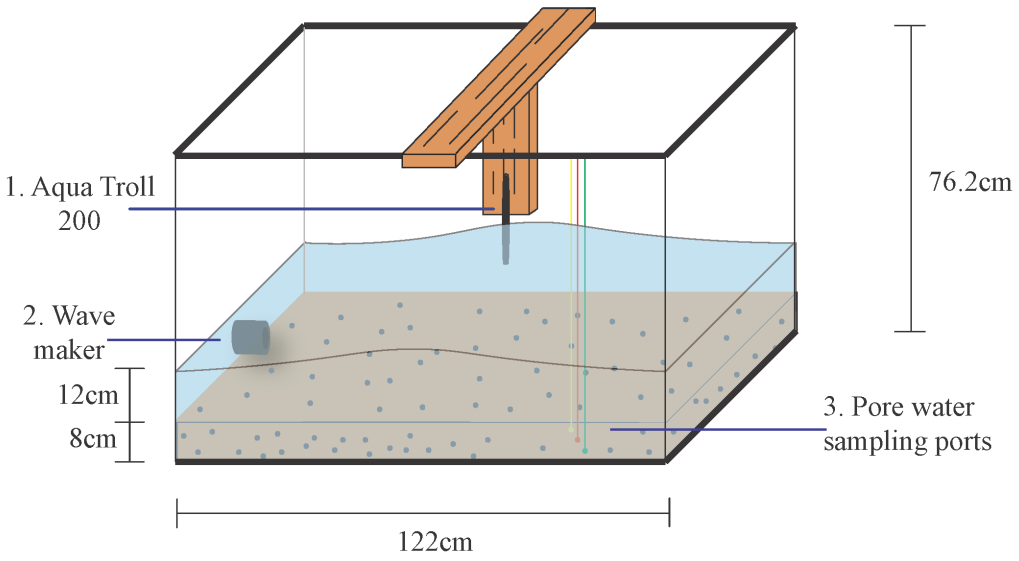

Figure 1: Tank setup for Wave Trial. Dimensions: 122 x 46 x 76 cm. Sediment Volume: ~1.5 ft^3. Water Volume: ~20 gal. 1) Aqua Troll 200 to monitor depth, temperature, and specific conductivity in surface water. 2) Wave maker (only present in wave trial). 3) Pore water samplers installed at 2, 4, and 6 cm below sediment water interface.

Figure 2: Tank setup for the Wave Trial. 1) Plastic sealant covering tank to minimize evaporation and splash over the course of the trials. 2) Aqua Troll 200 attached to board and suspended in the water column. 3) Pore water sampling ports installed at 3 depths below the sediment-water interface. 4) Wave maker control panel to adjuct speed, wavelength, and mode. 5) Wave maker.

Preliminary Trials

Multiple preliminary trials were conducted to finalize procedure and sampling schedule prior to final trials with MC-LR. Preliminary runs helped to plan the amount of conservative tracer to add, estimate the mixing time between surface water and pore water, and establish ideal wave settings for the tank so pore water samplers would not wash out. Preliminary trials were also used to assess whether sediment was sufficiently permeable to ensure a short experimental timescale (on the order of 2 days). In the first preliminary run, a mixture of sand and silt was used, resulting in an extremely slow equilibration time. This was problematic due to possible evaporative losses from the tank over time, which would interfere with chloride concentrations in surface water [*Turner and Townley,* 2006]. Sediments were sieved to exclude silt and fine sand in final trials, and the tank was also covered with plastic wrap to minimize evaporative losses, splash, and spills. The tank was also covered for the Wave Trial, and the system took about 24 hours to reach temperature equilibrium. Wave action created heat and the plastic wrap allowed for minimal escape. After 24 hours, temperature stabilized and held constant throughout the trial. Consequently, the system was allowed due time to equilibrate before trials began.

Sampling Methods

Pore water samplers were constructed of 16-mm interior diameter polyethylene tubing and positioned at depths of 2 cm, 4 cm, and 6 cm below the sediment-water interface at least 15 cm from the tank side walls. The sampling interval consisted of small holes drilled over a 10 cm interval of the tubing, which was covered in wear-resistant nylon mesh with a 50.8 μm opening to exclude sediment and prevent clogs. Mesh was secured to tubing via zip ties. Tubing ran to a 1.5 mm plastic elbow connector at the tank side wall and then up the side wall to a peristaltic pump. Pore water samples were pumped at an approximate rate of 1 L minute^{-1}. First, one tubing volume was discarded (3 mL). Next, 7 mL of pore water was pumped directly into an amber glass vial, and then 5 mL was transferred into a clarified polypropylene centrifuge tube with a polyethylene screw cap for analysis of chloride, while the remaining 2 mL was retained in the glass vial for MC-LR analysis.

Surface water samples were collected by dipping a gloved hand and amber glass vial directly into the tank, and filling the vial with approximately 7 cm of water from the top few centimeters of surface water. 5 mL of sample was then transferred to a centrifuge tube. In the Non-Wave Trial, surface water was slowly stirred three times before collecting each sample to ensure a well-mixed water column.

Samples for chloride analysis were stored in clarified polyethylene centrifuge tubes in the refrigerator, and samples for MC-LR ELISA analysis were stored in amber glass vials in a locked freezer on their sides.

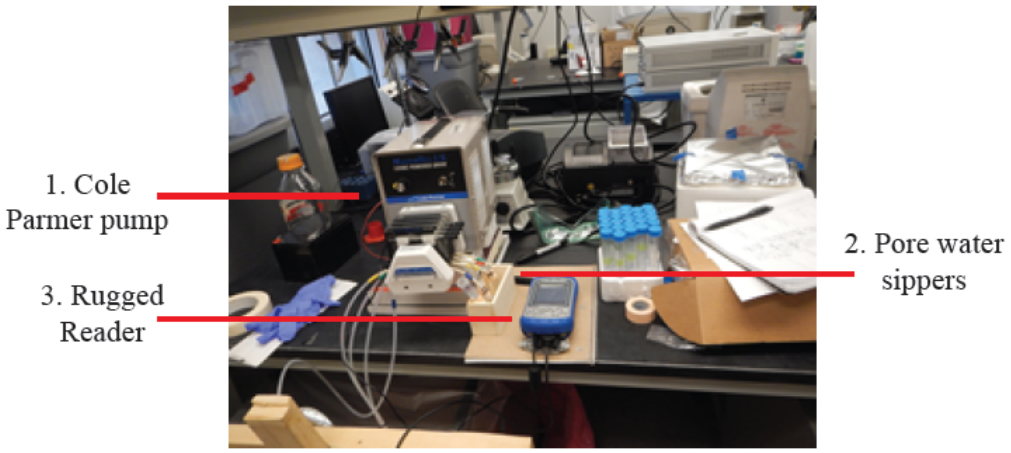

Figure 3: Sampling setup. Shown: 1) Cole Parmer pump, 2) pore water sippers pumping into discard centrifuge tubes, 3) Rugged Reader for the Aqua Troll 200

Sample Analyses

Surface and pore water samples were analyzed for chloride using a Dionex ICS-2100 ion chromatograph (IC) in Dr. Anne Carey and Dr. Berry Lyon's Environmental Geochemistry lab and using the methods of Welch et al. (1996). Surface water samples were diluted by a factor of 5.5, and pore water samples were diluted by a factor of 2.75. Several standards were included to span a wide range of chloride concentrations, and standards had less than 0.5% error. The error associated with pipetting during dilution was 0.2%. A subset of samples were diluted by mass as well to further eliminate dilution error.

Samples were analyzed for MC-LR using an Abraxis Microcystins/Nodularins (ADDA) ELISA kit (Product # PN520011). Undiluted samples were analyzed in duplicate, and standards were run to ensure accuracy. The lower detection limit for MC-LR was 0.15 ppb. All samples were analyzed at The Ohio State University.

Visualization of Benthic Exchange with Dye

A final visualization experiment was conducted to characterize patterns and rates of benthic exchange due to waves. In this experiment, blue food coloring was added to surface water, and photographs were taken at regular intervals for 3 hours. Sediment porosity and volume, water level, and wave amplitude were the consistent with the Wave Trial.

RESULTS

Benthic Exchange Visualization

The visualization experiment with dye shows that waves begin effectively mixing surface water with shallow pore water within minutes (Figure 4). Dye penetrates pore water more quickly in troughs of ripples compared to crests due to a differential pressure gradient at the sediment water interface. In the trough with the most rapid exchange, the dye reached 2 cm within 10 minutes, 4 cm within 30 minutes, and 5 cm within 3 hours. Dye extended to an average of 4cm across the tank within 3 hours (Figure 4f). In areas near crests where mixing was the slowest, dye reached >2 cm after 2 hours. After 3 hours, dye was present in 5 cm depth pore water, however dye concentrations were likely still gradient, with dye decreasing with depth into the sediment. It could be inferred from chloride data (Figure 5) that the full effective mixing depth of 6 cm would be reached, and concentration gradients would be equilibrated after 50 hours.

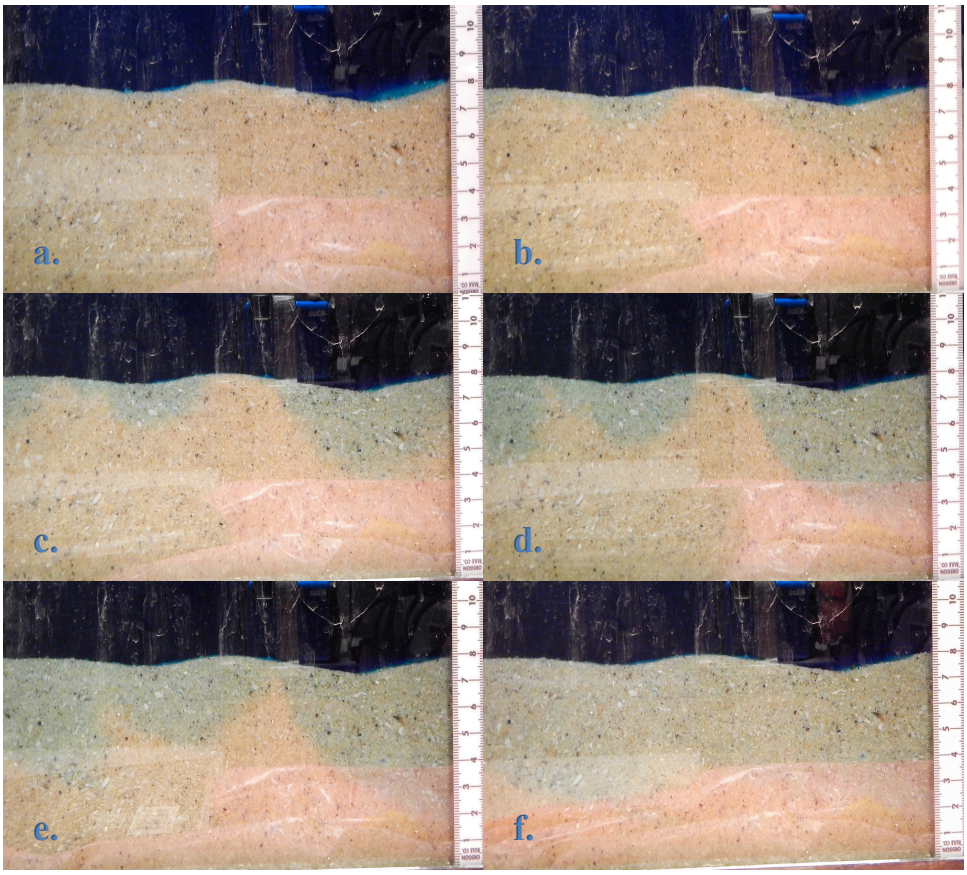

Figure 4: Visualization of benthic exchange in presence of wave action. a = start, b = 10 minutes, c = 30 minutes, d = 1 hour, e = 2 hours, f = 3 hours.

Conservative Transport in Wave and Non-Wave Trials

Water temperature, dissolved oxygen (DO), and pH in surface water were approximately constant over both trials (Table 1). Water temperature averaged 21 ± 1°C, pH was nearly neutral, and water volume variation due to evaporation was negligible (<20 mL or <0.05% of total water volume). Oxidation-reduction potential (ORP) values of 112-326 mV (Table 1) indicate an oxidizing environment, at least in surface water and overlying water. Oxidizing conditions are further supported by the significant presence of nitrogen as nitrate (Appendix IV). Additionally, significant concentrations of sulfate (>2.5 ppm) were present in surface water and all depths of pore water throughout the entire experiment (Appendix IV). Sulfate concentrations increased over time and with depth.

Changes in surface water chloride concentration over time reflect conservative mixing between surface water and pore water. For both trials, the expected maximum chloride concentration in surface water was 237 ppm, assuming complete mixing in surface water at early time. In the Wave Trial, the maximum observed chloride concentration was 236 ppm, and was reached within 30 minutes of chloride addition (Figure 4). The chloride concentration then declined asymptotically to nearly 208 ppm over a period of approximately 50 hours, with the fastest decline in the first 8 hours. Chloride concentrations in pore water at 2 cm below the sediment-water interface show a rapid increase, followed by a decline after 8 hours, and another increase at late time (Figure 5). Because this shallowest sampling port appeared to have washed out by the end of the study, concentrations measured at this port are unreliable and may sometimes reflect pore water concentrations, and at other times reflect surface water concentrations. At 4 cm below the sediment-water interface, the chloride concentration increased over 8 hours and attained a maximum concentration of 215 ppm (Figure 5). At 6 cm below the sediment-water interface, the chloride concentration reached a maximum of about 208 ppm after about 50 hours.

In the Non-Wave Trial, chloride concentrations in surface water peaked 7 hours after chloride addition at roughly 265 ppm (Figure 6). The late peak suggests that manual stirring was not fully effective at mixing the chloride and MC-LR solution in surface water. Chloride concentrations in surface water then slowly declined to 230 ppm over the duration of the trial. Chloride concentrations in pore water at 2 cm below the sediment-water interface increased steadily for the first 26 hours, reaching a maximum value of 200 ppm. At 4 cm, chloride concentrations increased gradually for the first 50 hours before stabilizing near a maximum of 200 ppm. Chloride concentrations at the deepest (6 cm) pore water sampler did not begin to increase until 50 hours. Subsequently, concentrations rose steadily through the end of the experiment. The final concentration at 6 cm was only 151 ppm, indicating that chloride was not fully mixed throughout the entire surface water and sediment column after nearly 100 hours (Figure 6).

The concentration of chloride in surface water, C_{Sw}, can be used to calculate an effective mixing depth d_{ex} in sediments assuming concentrations in pore water, C_{Pw}, are fairly uniform:

$$d_{ex} = [d_{Sw}(C_{Swo} - C_{Swf})] \div [\phi(C_{Swf} - C_{Pwo})] \qquad (1)$$

where the subscript o denotes initial time, ϕ is porosity, and d_{Sw} is the depth of overlying water. In the Wave Trial, I estimate an effective pore water mixing depth of 6.0 cm after 50 hours when chloride concentrations in surface water stabilized. In the Non-Wave Trial, the mixing depth is

3.5 cm after 50 hours and 5.7 cm after nearly 100 hours. Notably, pore water chloride concentrations in the Non-Wave Trial increased at the deepest port (6 cm) after only 50 hours (Figure 6), suggesting that pore water extraction during sampling may induce transport downward from the sediment-water interface near the sampling ports. Density differences associated with vertical salinity gradients may have also enhanced exchange in the absence of waves but only to a small extent: the density of initial surface water is 1.0005 g cm^{-3}, while the density of initial pore water is 1.0000 g cm^{-3}. The deeper exchange depths calculated in the Wave Trial and faster response times of chloride in pore water clearly demonstrate the role that waves play in solute exchange across the sediment-water interface. For comparison, the time to reach 50% of maximum concentration at 4 cm depth is 1.5 hours with waves and 36 hours without waves.

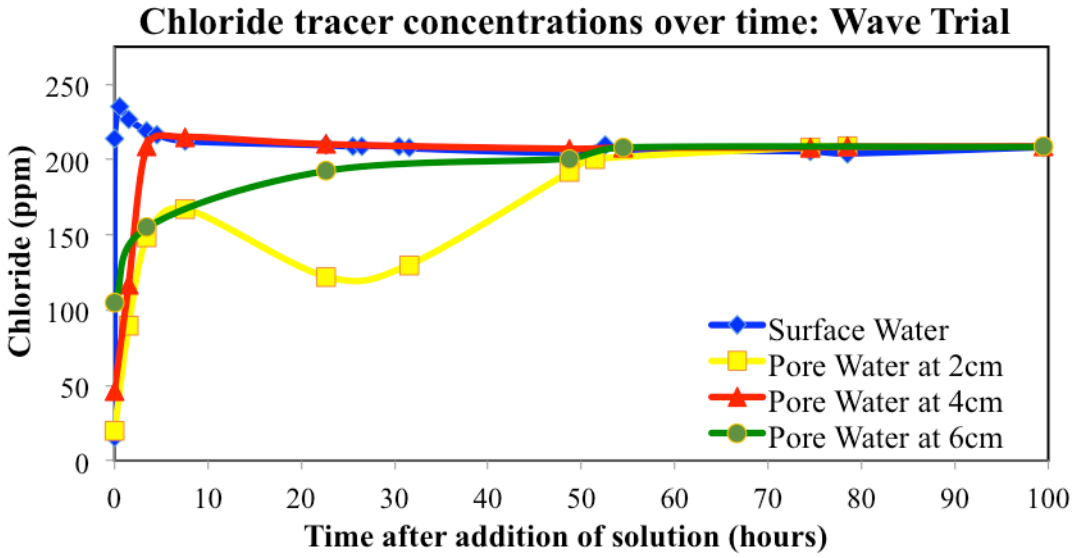

Figure 5: Chloride concentration in surface and pore water over time for the Wave Trial.

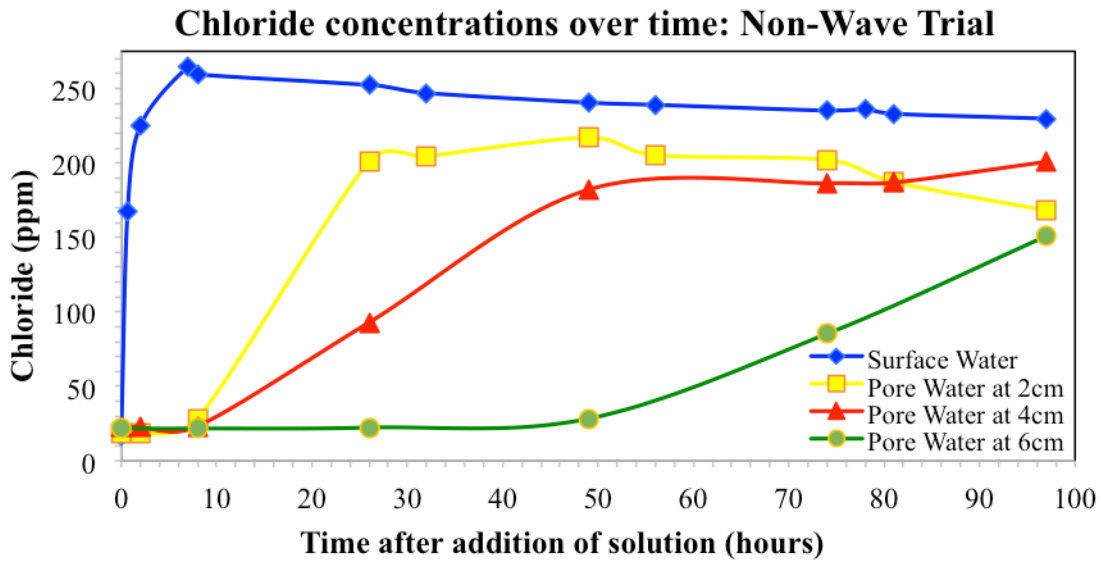

Figure 6: Chloride concentration in surface and pore water over time for Non-Wave Trial.

	Time/Date	Temp (°C)	DO (%L)	DO (mg L^{-1})	ORP (mV)	pH	C (mS cm^{-1})	Water Level (cm)
Start Non-Wave:	2/4/16 9:02 am	21.1	80.3	7.00	326.1	7.11	1.10	19.7
End Non-Wave:	2/8/16 10:00am	20.9	80.2	7.03	265.8	7.60	0.81	19.6
Start Wave:	2/18/16 9:26am	21.0	95.0	8.28	112.1	7.27	0.87	20.0
End Wave:	2/22/16 1:00pm	21.6	93.7	8.14	206.5	7.25	0.74	19.9

Table 1: Surface water temperature, dissolved oxygen, oxidation-reduction potential, pH, conductivity, and as measured by YSI electrodes. Water level measurements read off of an adhesive ruler, which was secured to the tank wall (as seen in Figure 4).

MC-LR Transport

In the Wave Trial, MC-LR concentrations in surface water behaved similarly to the conservative chloride at early time but then continued to decline over the course of the trial (Figures 7 and 8). Initial concentrations in surface water and pore water were below the detection limit prior to MC-LR addition. The expected maximum MC-LR concentration in surface water was 11.5 ppb, assuming complete mixing in surface water. The maximum observed concentration in surface water was only 1.19 ppb, which was reached within the first 30 minutes of the experiment (Figure 7). MC-LR concentrations in surface water decreased rapidly over the first half hour to approximately 0.97 ppb and then continued to decrease more gradually for the remainder of the trial to 0.36 ppb after approximately 100 hours. This steady decline relative to chloride (Figure 8) suggests measurable, non-conservative removal of MC-LR over the course of the trial. After thorough mixing (50 hours), surface water MC-LR concentrations fell 52%, while chloride concentrations fell only 12% (Figure 8). At the end of the trial, surface water MC-LR concentrations fell by 70%.

In shallow pore water at 2 cm depth, MC-LR concentrations increased to 0.35 ppb within 4 hours and then remained nearly constant over the remainder of the trial (Figure 7). MC-LR concentrations at this depth are considered unreliable based on observed erosion around the sampling port due to wave action. MC-LR concentrations at 4 cm below the sediment-water interface reached a maximum of 0.66 ppb after about 4 hours and then gradually declined over the remainder of trial, similar to MC-LR in surface water (Figure 7). Similarly, MC-LR concentrations 6 cm below the sediment-water interface reached a maximum of 0.49 ppb around 4 hours and then declined gradually. Concentrations in deep pore water (6 cm) were consistently less than concentrations in surface water and shallower pore water (4 cm), with the exception of the final sample at 100 hours, where the concentrations were almost equal. Throughout the entire trial, normalized MC-LR concentrations were less than normalized chloride concentrations at all pore water depths (Figure 8), indicating highly non-conservative transport.

Three preliminary MC-LR samples were run from the Non-Wave Trial (Appendix III). These samples were from surface water within the first 26 hours of the trial, when MC-LR concentrations should presumably have ranged from intermediate to maximum values. All three samples were surprisingly low in concentration (<0.3 ppb), and therefore concentration changes throughout the remainder of the experiment were likely below the error of the ELISA kit. Consequently, no additional samples were run from this trial. The surface water was noticeably more turbid at the start of the Non-Wave Trial relative to the Wave Trial. It is possible that MC-LR sorbed strongly to suspended sediments almost immediately upon addition to the tank, which would explain the lower-than-predicted maximum MC-LR concentrations in both trials, and particularly low concentrations in the Non-Wave Trial.

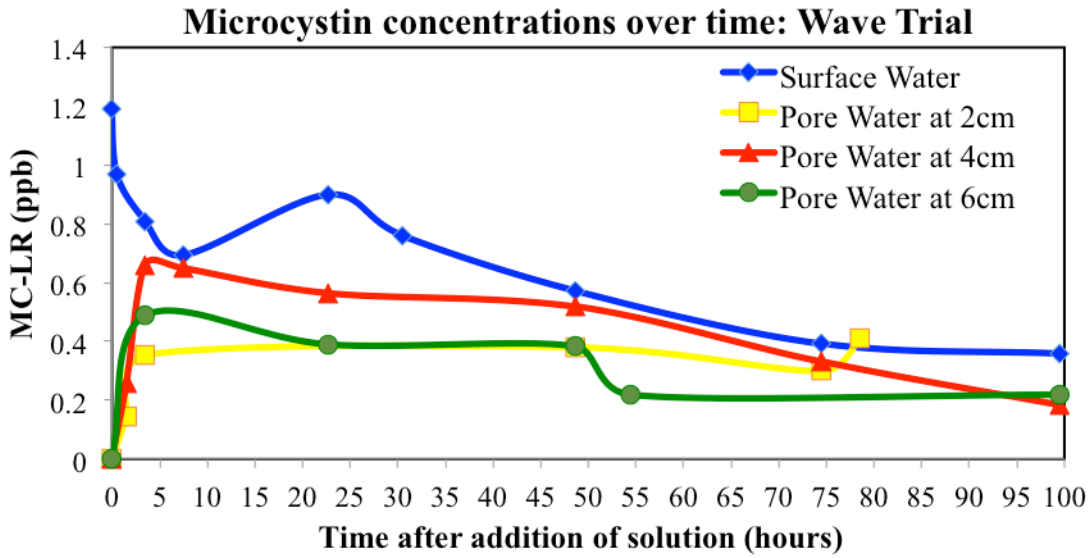

Figure 7: MC-LR in surface water and pore water over time for the Wave Trial. Note: 100 hour sample of pore water at 2 cm was not analyzed due to observed erosion near sampling port due to wave action.

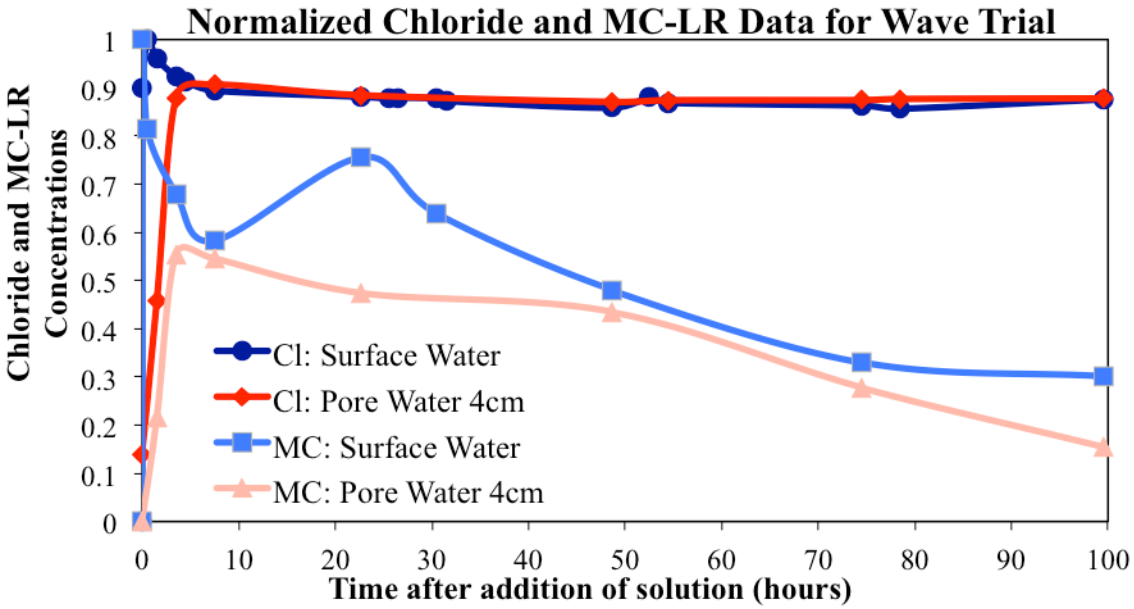

Figure 8: Normalized (dimensionless) chloride and MC-LR data for the Wave Trial. A value of 1 represents the maximum concentration attained in surface water for chloride or MC-LR. A value of 0 represents the initial concentration of chloride or MC-LR in surface water prior to the addition of solute.

DISCUSSION

As predicted, wave pumping rapidly increases the rate of benthic exchange relative to stagnant conditions, and MC-LR is effectively removed from both surface water and sediments. Attenuation mechanisms for MC-LR in the tank include dispersive mixing between surface water and pore water and reactions such as sorption and biodegradation. Although some photodegradation may have occurred in surface water, photodegradation would not occur in pore water, where MC-LR transport was also highly non-conservative. It is therefore assumed that the effects of photodegradation are small and interpretations are focused on dispersion, sorption, and biodegradation processes.

The effects of dispersive mixing between surface water and pore water on MC-LR concentrations can be determined from chloride concentrations. Specifically, chloride in surface water experienced a decrease of 12% after 100 hours due to dispersion, while MC-LR experienced a decrease of 70%. Of the 70% decrease, I assume 12% is due to dispersive mixing between surface water and pore water, while the remaining 58% is due to non-conservative removal, most likely from combined effects of sorption and biodegradation. The total estimated decline of 70% is a minimum, as calculations suggest that the maximum MC-LR concentration in surface water should have been much greater. The maximum conservative chloride concentration in surface water was approximately equal to the expected concentration from calculations, while the maximum observed MC-LR concentration was approximately 90% less than the expected concentration. Assuming that the maximum MC-LR concentration was indeed 11.5 ppb as predicted instead of 1.2 ppb as analyzed from a sample collected within minutes of MC-LR addition, the total loss is 97%. Furthermore, most of this loss occurred almost immediately upon addition of the MC-LR solution. The solution that was added to the tank was not analyzed for MC-LR to check for errors in preparation, but will be analyzed in the future.

Sorption

The rapid loss of MC-LR from surface water and sediments at early time (minutes to hours) may be due to sorption. MC-LR has been shown to sorb moderately to sediments in batch experiments [*Chen et al.,* 2008*; Song et al.,* 2014*; Wu et al.,* 2011]. In a closed system such as a tank, sorption rates should decline over time as the sorption capacity of suspended and bed sediments are exhausted. If initial MC-LR concentrations in surface water were in fact near 11.5 ppb, and fell to 1.2 ppb within minutes, sorption to suspended sediments may be the only mechanism fast enough to explain the removal. Sorption may also help explain low MC-LR concentrations in pore water at early time. As benthic exchange delivers MC-LR to fresh sediments, sorption may remove a portion of the MC-LR. For example at 4 cm depth, MC-LR concentrations only rose to 60% of the expected value based on chloride after 4 hours (Figure 8) and had declined to 17% after 100 hours.

If sorption is an important mechanism for rapid removal of MC-LR, more suspended matter in the water column should facilitate greater removal. Indeed, surface water in the Non-Wave Trial was visually cloudier than surface water in the Wave Trial, and maximum observed MC-LR concentrations in surface water in the Non-Wave Trial were lower (Appendix III). The high turbidity in the Non-Wave trial is somewhat counterintuitive, since wave action would be expected to increase turbidity. However, the Non-Wave Trial was conducted first, so the sediment likely contained more fine-grained particles. Most of these fine-grained particles were

likely removed during the multiple tank flushings between the Non-Wave Trial and Wave Trial. In addition, the Non-Wave Trial began less than 24 hours after water was added to the tank, which left insufficient time for all suspended particles to be removed by settling.

MC adsorption can occur in the presence of clays, iron oxides, and naturally suspended sediments [*Lee and Walker,,* 2011*; Wu et al.,* 2011]. Although the vast majority of sediment was medium sized pool filter sand, and natural Lake Erie sediment was sieved twice to remove as much silt-clay sized particles as possible, fine particles were still mobilized into the water column. It would be helpful in the future to test for turbidity in the surface water using a turbidity meter, and analyze for composition of fine particles using the XRF or SEM. In a batch study by Morris et al. *[2000],* more than 80% of MC-LR was removed via adsorption to clay particles. This is consistent with results of both trials, and should be further investigated.

Degradation

Biodegradation has been shown to play a major role in the attenuation of MC-LR in the presence of oxygen and microbially active sediments [*Wu et al.,* 2000], and may have contributed to decay of MC-LR in both surface water and sediments at later times. Biodegradation often does not occur immediately, while sorption effects typically lessen rapidly in a closed system. Hence, a reasonable interpretation is that attenuation of MC-LR after approximately 50 hours is mainly due to biodegradation. This late-time attenuation is much slower than early-time attenuation (Figure 8), which may suggest that biodegradation is less important than sorption in removing MC-LR from surface water and sediments overall.

It is unclear whether biochemical conditions in the sediment were particularly favorable for biodegradation. Although I was not able to measure dissolved oxygen in pore water within the tank, the high permeability and benthic exchange rates in sediments likely contributed to efficient oxygen transfer across the sediment-water interface. Microbial activity of the sediments is unknown, but less than 10% of the total sediment bed consisted of natural lake sediment, and organic matter content appeared to be low. Furthermore, the sediment was not analyzed for biomass abundance or phylum. More biogeochemical analyses in surface water and sediments would be useful for understanding biodegradation behavior.

Benthic Exchange Rates and Dispersion

Conservative mixing of surface water and pore water played a relatively small role in reducing MC-LR concentrations, explaining only 12% of the observed decrease. However, benthic exchange is still important for delivering MC-LR to sediments where non-conservative processes such as sorption and degradation can occur. Not surprisingly, these experiments showed that benthic exchange was faster and deeper in the Wave Trial than the Non-Wave Trial. After a thorough mixing period of 50 hours in the Wave Trial, the estimated exchange depth into the sediments was 6.0 cm, while it was only 3.5 cm in the Non-Wave Trial. Similarly at 4 cm depth, the time to reach 50% of the maximum chloride concentration was only 1.5 hours in the Wave Trial and 34 hours in the Non-Wave Trial (approximately 30 times faster). This study suggests that wave action can drastically increase the volume of sediment that interacts with the surface water in coastal environments and thereby increase the total potential for MC-LR mass removal. Wave action also increases the rate of sediment-water interactions and therefore decreases the time required for removal.

Benthic exchange increases with wave size and rippled bed topography [*Precht and Huettel.,* 2003]. Waves create a sinusoidal pressure gradient along the sediment-water interface that drives surface water to flow into ripple troughs and out of ripple crests [*Rutherford et al.,* 1995]. This explains why dye penetration was deeper beneath bedform troughs in my visualization experiment (Figure 4). Environments with both waves and rippled beds are likely to be more effective at MC-LR removal, all other factors held constant.

Real World Implications

These trials show that many chemical processes associated with the interaction of water and sediments causes rapid attenuation of MC-LR plumes in shallow, high-energy settings. Suspended sediments in the water column provide opportunities for sorption and biodegradation. Benthic exchange provides opportunities for sorption, biodegradation, and dilution. Conditions in the tank were optimized for benthic exchange. Near natural coasts, benthic exchange may be lower, depending on porosity and permeability, depth of the water column, and energetic conditions. At lower porosities, benthic exchange would likely be slower, but sediment surface area available for sorption might be greater. This tradeoff requires further investigation and is important for understanding MC-LR fate in systems like Lake Erie, where there is more glacial till and clay-sized particles than in the tank sediment. It is also important to note that sediment-water interactions are greater in shallow coastal waters than deep lake waters. The strongly non-conservative transport behavior observed in my tank experiments may not occur in deep water, where only a small fraction of the water column interacts with the lakebed.

Results show that while benthic exchange does play a role it the attenuation of MC-LR, sorption to suspended sediments may be more crucial for early removal of MC-LR after release from HABs. Additionally, sorption may be strongly dependent on turbidity. Specifically, in shallow, energetic coastal settings, it is possible that the majority of MC-LR is sorbed almost immediately, and peak concentrations are significantly reduced due to the presence of sediments. Consequently, wind and wave conditions, which influence turbidity, may be key factors in predicting peak MC-LR concentrations near coasts. In deep or calm settings with less turbidity, natural attenuation of MC-LR may be relatively low, and potential peak MC-LR concentrations may be much greater, other factors remain constant.

Future efforts should focus on the reversibility of MC-LR sorption. Desorption from sediments could lead to slow, persistent MC-LR release from sediments back to surface waters, particularly under certain shifts in water chemistry or temperature. In light of warming lake temperatures and anthropogenic disturbances to lake chemistry, it is particularly important to understand potential sources of MC-LR from sediments to lake water.

Conclusions

Wave action greatly enhances solute transfer between surface water and sediments, and MC-LR is rapidly removed in the presence of waves. MC-LR concentrations rapidly decrease within minutes to hours in surface water, likely resulting from sorption to suspended and bed surface sediments. Over timescales of hours to days, MC-LR continues to decline, likely due to both biodegradation and continued sorption in both surface water and sediments. These results from a controlled tank study suggest that sorption and degradation play a major role in MC-LR attenuation in wave-dominated nearshore environments. The effects of sorption and degradation in deep water settings are unclear, especially since MC-LR removal rates were not assessed under stagnant conditions. Suspended sediment was not controlled in tank trials, leading to variations in turbidity. Results suggest that MC-LR removal by sorption to suspended sediments may be a more significant factor in overall attenuation than previously believed. Repeat tests are planned for the future to isolate the effects of turbidity, and better assess MC-LR fate in deep water settings.

RECOMMENDATIONS FOR FUTURE WORKS

These experiments raise a variety of questions about MC-LR fate in shallow waters. The low concentrations of MC-LR, particularly at early time, suggest that sorption to sediments may be a bigger factor in the overall attenuation than previously believed. In the future, the concentrated chloride-MC-LR solution should be sampled before addition to the tank to rule out the possibility of error in expected MC-LR concentrations at early time. Total surface area of particles should also be considered in order to quantify potential binding sites for MC-LR, and a more in-depth sediment chemistry and grain size analysis should be conducted. In order to quantify the fraction of MC-LR adsorbed to bed sediments, a grab sample or core should be collected at the end of experiments, and MC-LR should be extracted and measured. A sample of the top several centimeters of sediment was collected at the end of the Wave Trial, but the sample has not yet been analyzed because the method for extracting MC-LR from sediment is still under development. Once methods are developed, it will be possible to quantify the mass of MC-LR sorbed to the bed but not to suspended sediments. To understand relationships between suspended sediment concentrations and MC-LR concentrations, batch experiments may be more useful. Another way to isolate the effects of sediment on MC-LR fate would be to compare two trials with and without sediment, but with similar wave conditions. I recommend conducting another tank experiment with the same waves but no sediment over an extended period of time with consistent surface water sampling. MC-LR attenuation rates and maximum concentrations can then be compared with and without sediment under the same wave and light conditions to test the effects of sediment-water interactions.

Future efforts should focus on quantifying sorption of MC-LR to sediments using other experimental approaches. Batch studies should be run involving small volumes of sediment and water to measure the amount of sorption under different turbidities and consistent mixing conditions. Field experiments that involve both sediment and water samples could also provide valuable insights into MC-LR fate. Finally, reversibility in MC-LR sorption should be investigated under a range of water conditions to determine whether sediments may become a source of MC-LR to surface waters.

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APPENDICES

Appendix I: Standard Operating Procedure

THE OHIO STATE UNIVERSITY

STANDARD OPERATING PROCEDURE

January 13th, 2016

CAUTION:

Microcystin-LR (MC-LR) is a harmful cyclic heptapeptide hepatotoxin and contact with exposed skin should be avoided as much as possible. Gloves should be worn while handling MC-LR, and must be removed before touching shared surfaces including door handles, light switches, etc. Be sure to always remove one glove before entering the hallway so as not to contaminate clean space. When in solution, MC-LR can be disposed of down the sink. Sediment that has been in contact with MC-LR must be put in plastic buckets obtained from Dr. Jiyoung Lee's lab and disposed of through OSU Environmental Health and Safety.

REAGENTS:

Chloride salt tracer: Dissolve 26 g of NaCl table salt (about 15.8 g Chloride) in a 1000 mL glass beaker filled with 1000 mL RO water. Must allow to sit for at least 30 minutes prior to addition to aquarium tank to ensure dissolution into solution.

MC-LR stock solution: MC-LR stock powder is a purified version of the toxin, extracted from lysed Microcystis cells. It is stable in powder form for up to 1 year and in water-based solution for up to 6 months. MC-LR stock solutions can be prepared on benchtop and stored in designated refrigerator found in the laboratory. To prepare solution, mix 0.77 mg of the MC-LR stock powder into the 1000 mL *glass* beaker (adsorbs to plastics) that already contains the salt tracer using a pipette. A 0.77 ppm MC-LR stock solution will give the tank surface water (18 gal) an initial concentration of 11 ppb. Note: Microcystin stock is not to leave Dr. Jiyoung Lee's lab (rooms 451 and 455 in The College of Public Health) according to the Transmission Control Protocol (TCP).

MATERIALS AND INSTRUMENTS:

Experiments are conducted within a 110-gallon glass aquarium tank. The tank is filled up approximately 8 cm from the base with about 12 gallons of aggregate sediment. Sediment aggregate contains primarily pool filter #20 silica sand (0.420 mm grain size). A small volume (~10%) consists of sediment from Lake Erie, sieved with #304 mesh (178 μm opening). The tank is filled and drained between experiments using an Aqueon brand Aquarium Water Changer. The Aquarium Water Changer consists of an 11-inch gravel tube connected by 25 feet of flexible tubing to a water flow sink attachment. It works by turning on the sink and setting the water flow control valve to either fill or empty the tank.

Pore water samples are collected with a Cole-Parmer pump (model number: 07533-20, Masterflex L/S 12VDC powered drive). Masterflex neoprene food grade tubing is used directly with pump and pump cartridge and is connected to extensions of 1/16 inch interior diameter (ID)

polyethylene tubing. Ideal setting for model are occlusion set between #3 and #4 (as indicated on small cartridge) and speed dial set at 1/3 of the way between 0 and 1 on the pump, resulting in a sampling rate of about 20 rpm and 1 mL minute^{-1}. Pore water samples are collected at approximately 2 cm, 4 cm, and 6 cm below the sediment-water interface. Tubing runs along the wall of the tank and then makes a 90 degree turn into the sediment via a 1/16 inch plastic elbow connector and projects out about 10 inches toward the tank center. Small holes were drilled into the last 4 inches of the 10 inch long sections of tubing, and covered in wear-resistant nylon mesh with 0.0020 inch opening to keep sediment out and prevent clogs. Mesh is secured to tubing via zip ties. Surface water samples were collected by hand-dipping an amber glass vial into the tank, and filling approximately 7 cm.

Waves are created using Jebao wave maker WP-25. The control panel is secured to the exterior wall of the aquarium tank. The wave maker pump head should be attached magnetically about 10cm above the sediment surface. Adjustments can be made using the control panel, including 6 possible "modes," 3 speeds, and wave duration. This instrument is only used in Experiment 1: Wave Trial. Ideal settings are W1, S3, and wave duration as marked on control panel. Ideal wave settings were determined via trial and error dependent on tank size and water volume present. W1 mode features adjustable pulses at maximum flow rate. This creates a consistent, oscillating current and wave height of approximately 2 cm in 12 cm of surface water. Speed S3 is at 50% of the maximum speed of the wave maker pump.

An Aqua Troll 200 (In-Situ Inc., S/N 398675) is used to monitor conductivity, temperature, and water level in the tank during Experiments 1 and 2. The Aqua Troll 200 is secured to a wooden 2"x4" and placed in the water column for the duration of both experiments. Two wooden 2"x4"s are connected in a "T" shape and clamped in place to rest on top the tank. The Aqua Troll 200 is connected to a RuggedReader (In-Situ, Inc., model number AR-RR2) to visually monitor and record parameters.

A YSI Professional Plus is used at occasional times throughout experiments to record redox potential, dissolved oxygen, conductivity, and pH in surface water.

PROCEDURE:

I. Tank Preperation for Experiment 1: Wave Trial

A. 24 hours before experiment
    1. Before adding water, bury the 3 pore water sippers at 2 cm, 4 cm, and 6 cm below the sediment surface. They should be positioned about 32 inches away from the wave maker.
    2. Ensure sediment bed is relatively level, then fill with about 18 gallons RO water using the aquarium hose. Ensure water level is at 12 cm above sediment and Jebao wave maker is approximately 10 cm above sediment and in the center of the tank, mode is set to W1, speed is set to S3, and wavelength dial is in the marked position seen on the control pad. Turn on wave maker and allow it to run for about 24 hours before beginning the experiment to allow surface water temperature to approach a steady state and allow suspended sediment disrupted by water addition to settle.

Note: Monitor tank for a few minutes once wave maker is turned on and ensure that consistent waves are being made with minimal sediment disruption. Adjustments with the wavelength dial and positioning of wave maker may be required.

3. Cover tank loosely with plastic wrap to prevent evaporation, but still allow for an influx of oxygen and release of heat.

B. Immediately before experiment
    1. Secure Aqua Troll 200 to wood board and make sure it is positioned so the tip is about halfway in the water column. Tip should never be in contact with the sediment or exposed above water while recording. Turn on RuggedReader logger and record temperature (degrees C), conductivity, and water level (cm) every 25 minutes.
    2. Position pump near pore and surface water sippers, make sure amber glass vials and Falcon brand centrifuge tubes are cleaned and marked with labeling tape.
    3. Ensure waves are still consistent in wavelength. Bedforms should be developed on sediment surface.

II. Sampling procedure

A. Sample vessel prep and labeling
    1. All 144 amber glass vials should be marked at 7 mL and centrifuge tubes should have labeling tape on them before starting experiment.
    2. Label every sample upon collection with date, time, and sample name. *Note:* Pore samples should be named by port color and sampling time. Color: red=R, green=G, and yellow=Y (color key found below).
    3. One "*round*" of sampling will include both a centrifuge tube and amber glass vial filled with designated water volume (5 mL and 2 mL, respectively) from each pore water port and the surface water sample (total of 8 filled vials per *round*). First fill amber tube with 7 mL, then transfer 4-5 mL to centrifuge tube, leaving 2-3 mL in amber tube.

B. Pore water samples
    1. Pore water sippers will be installed and marked at 2 cm (yellow), 4 cm (red), and 6 cm (green) below sediment surface. Pore water will be sampled using the Cole-Parmer Masterflex L/S 12VDC pump. Pump sampling speed is 1/3 of the way between 0 and 1 on the speed dial and will be marked on the pump.
    2. To ensure stagnant water is not being sampled, pump 3 mL from *each* port into a used centrifuge tube (marked) and discard in sink or bucket.
    * If above 1ppb MC-LR, must properly dispose in biohazard buckets.
    3. Pump 7 mL of water from each port into corresponding amber vial. Transfer 5 mL of sample into centrifuge tube for chloride analysis on IC. Secure cap, label, and place centrifuge tube in refrigerator.
    4. Amber glass vial should still contain 2 to 3 mL of sample (for MC-LR analysis by ELISA). Secure cap, label, and place in freezer on its side.

C. Surface water samples
    1. Surface water will be sampled using the Cole-Parmer pump Masterflex L/S 12VDC pump. Pump sampling speed is 1/3 of the way between 0 and 1 on the speed dial when sampling at same time as pore water. When being pumped

alone, ensure that the pore water cartridges are set to occlusion >5 so that only surface water is being sampled. Maximum speed can be used.
Note: For the first surface water sample (immediately after chloride and MC-LR solution addition) sample by hand. Make sure to wear a glove to avoid exposure.

2. To ensure stagnant water is not being sampled, pump 2 mL meter^{-1} of tubing into a used centrifuge tube (marked) and discard in bucket.
   * If above 1 ppb MC-LR, must properly dispose in biohazard buckets.
3. Pump 7 mL of water into amber vial. Transfer 4 to 5 mL of sample into clean syringe attached to 0.4 μm nylon filter. Filter syringe contents into centrifuge tube (for chloride analysis on IC). **Secure cap, label, and place centrifuge tube in refrigerator.**
4. Amber glass vial should still contain 2 to 3 mL of unfiltered sample (for MC-LR analysis by ELISA). Secure cap, label, and place in freezer on its side. These samples should not be filtered as it could interfere with microcystin concentration.

D. Sampling schedule for Experiment 1: Wave Trial

Note: Sample schedule is subject to speed up or slow down based on visual monitoring of surface water conductivity data. Official schedule attached to tank during experiments.

-YSI measurement should be taken with each sample time.

Sampling Schedule for Experiment 1: Wave Trial						
t (hr after start)	Date	Time	SW	2 cm	4 cm	6 cm
0 (before tracer)	2/18/16	8:55 AM	X	X	X	X
0 (after tracer)	2/18/16	9:00 AM	X			
1	2/18/16	10:00 AM	X			
2	2/18/16	11:00 AM	X	X	X	X
3	2/18/16	12:00 PM	X			
4	2/18/16	1:00 PM	X	X	X	X
5	2/18/16	2:00 PM	X			
7	2/18/16	4:00 PM	X	X	X	X
9	2/18/16	6:00 PM	X			
23	2/19/16	8:00 AM	X	X	X	X
26	2/19/16	11:00 AM	X			
27	2/19/16	12:00 PM	X			
28	2/19/16	1:00 PM	X			
30	2/19/16	3:00 PM	X			
31	2/19/16	4:00 PM	X			
32	2/19/16	5:00 PM	X	X	X	X
49	2/20/16	10:00 AM	X	X	X	X
53	2/20/16	2:00 PM	X			
55	2/20/16	4:00 PM	X	X	X	X
75	2/21/16	12:00 PM	X	X	X	X
79	2/21/16	4:00 PM	X	X	X	X
100	2/22/16	1:00pm	X	X	X	X

* YSI measurements for surface water conductivity, dissolved oxygen, pH, redox potential, and temperature should be taken with each round of sampling.

E. Sampling Schedule for Non-Wave Trial

Note: Sample schedule is subject to speed up or slow down based on visual monitoring of surface water conductivity data. Official schedule attached to tank during experiments.

-YSI measurement should be taken with each sample time.

Sampling Schedule for the Non-Wave Trial						
t (hr after start)	Date	Time	SW	2 cm	4 cm	8 cm
0 (before tracer)	2/4/16	8:55 AM	X	X	X	X
0 (after tracer)	2/4/16	9:02 AM	X			
2	2/4/16	11:00 AM	X	X	X	X
4	2/4/16	1:00 PM	X			
7	2/4/16	4:00 PM	X			
8	2/4/16	5:00 PM	X	X	X	X
26	2/5/16	11:00 AM	X	X	X	X
28	2/5/16	1:00PM	X			
30	2/5/16	3:00PM	X			
32	2/5/16	5:00PM	X	X	X	X
49	2/6/16	10:00AM	X	X	X	X
54	2/6/16	3:00PM	X			
56	2/6/16	5:00PM	X	X	X	X
74	2/7/16	11:00 AM	X	X	X	X
76	2/7/16	2:00 PM	X			
81	2/7/16	6:00 PM	X	X	X	X
97	2/8/16	10:00 AM	X	X	X	X

*YSI measurements for surface water conductivity, dissolved oxygen, pH, redox potential, and temperature should be taken with each round of sampling.

III. Start of Wave Trial

A. Set the Aqua Troll 200 to take pressure, temperature, and conductivity data every 15 minutes throughout the experiment and position it in the tank.
B. Add chloride tracer and MC-LR solution to the tank after taking the first samples. Solutions should be added simultaneously, and distributed as evenly as possible over the tank.
C. Make sure tank remains covered loosely with plastic wrap to prevent evaporation, but still allow for an influx of oxygen and release of heat.
D. Follow sampling procedure and schedule for duration of experiment.
E. Experiment does not need to be constantly monitored, however it should be checked every so often to make sure waves are still constant, pore water sippers remain in place, dissolved oxygen and temperature in surface water are relatively constant, and evaporation does not appear to be influencing water levels.

IV. End of Wave Trial/ Prep for Non-Wave Trial

A. When experiment is over and system has equilibrated, turn off and remove wave maker, Aqua Troll 200, and uninstall pore water sampling ports from the sediment. Pump fresh RO water through tubing and into amber glass vials to test for sorption. Then, pump tap water through pore water sippers several times before next experiment to cleanse mesh and tubing.
B. Empty water from the tank using the Aqueon Aquarium Water Changer. Due to the large water to microcystin ratio, disposal in the sink is permitted. However, run sink at the same time to dilute.
C. Refill the tank using the Aquarium Water Changer and tap water, mix sediment around using a shovel to suspend and flush the sediment, and then empty tank. Repeat 2-3 times. Note: tap water is fine for flushing the tank between experiments, however RO water should be used when filling the tank a final time before the start of an experiment.
D. While tank is empty, wipe down walls of tank with warm water and reinstall pore water sippers at 2 cm, 4 cm, and 6 cm.
E. Refill the tank a final time with 18 gal of RO water for use in Non-Wave Trial and allow to settle for a few hours before the experiment begins (preferably overnight).

V. Procedure for Non-Wave Trial

A. Set the Aqua Troll 200 to take pressure, temperature, and conductivity data every 25 minutes throughout the experiment and position it in the tank.
B. Add chloride tracer and MC-LR solution to the tank after taking control samples. Solutions should be added simultaneously, and distributed as evenly as possible over the tank.
C. Make sure tank remains covered loosely with plastic wrap to prevent evaporation, but still allow for an influx of oxygen and release of heat.
D. Follow sampling procedure and schedule for duration of experiment.

Note: Wave maker will not be used in this experiment. Instead, very slowly stir surface water in tank with one single circular rotation before each sample interval so as to mix surface water but create minimal exchange with pore water.

VI. Clean-up for Non-Wave Trial

A. When experiment is over and system has equilibrated, turn off and remove Aqua Troll 200, and uninstall pore water sampling ports from the sediment.
B. Empty water from the tank using the Aqueon Aquarium Water Changer. MC-LR in solution can be disposed of down the sink.

Appendix II: Chloride and MC-LR Data for Wave Trial

Sample Location	Hour(s) after start	Chloride (ppm)	MC-LR (ppb)
SW	0	16	*0.08
SW	0	214	1.19
SW	1	236	0.97
SW	2	227	-
SW	4	219	0.81
SW	5	216	-
SW	8	212	0.69
SW	23	210	0.90
SW	26	209	-
SW	27	209	-
SW	31	209	0.76
SW	32	208	-
SW	49	205	0.57
SW	53	209	-
SW	55	207	-
SW	75	206	0.39
SW	79	204	-
SW	100	208	0.36
PW 2cm	0	20	*0.08
PW 2cm	2	89	0.15
PW 2cm	4	148	0.35
PW 2cm	8	167	-
PW 2cm	23	122	-
PW 2cm	32	13	-
PW 2cm	49	192	0.38
PW 2cm	52	200	-
PW 2cm	75	208	0.30
PW 2cm	79	209	0.41
PW 4cm	0	47	*0.07
PW 4cm	3	117	0.26
PW 4cm	4	209	0.66
PW 4cm	8	215	0.65
PW 4cm	23	210	0.56
PW 4cm	49	207	0.52
PW 4cm	55	208	-
PW 4cm	75	208	0.33
PW 4cm	79	209	-
PW 4cm	100	209	0.18
PW 6cm	0	105	*0.07
PW 6cm	4	155	0.49
PW 6cm	23	193	0.39

PW 6cm	49	201	0.38
PW 6cm	55	208	0.22
PW 6cm	100	209	0.22

SW=Surface Water, PW=Pore Water, *value is below detection limit and unreliable

Appendix III: Chloride and MC-LR Data for Non-Wave Trial

Sample Location	Hour(s) after Start	Chloride (ppm)	MC-LR (ppb)
SW	0	16	*0.07
SW	0	167	-
SW	2	225	-
SW	7	265	-
SW	8	260	*0.27
SW	26	252	*0.23
SW	32	247	-
SW	49	241	-
SW	56	239	-
SW	74	235	-
SW	78	236	-
SW	81	233	-
SW	97	230	-
PW 2cm	0	19	-
PW 2cm	2	19	-
PW 2cm	8	28	-
PW 2cm	26	201	-
PW 2cm	32	205	-
PW 2cm	49	217	-
PW 2cm	56	205	-
PW 2cm	74	202	-
PW 2cm	81	187	-
PW 2cm	97	168	-
PW 4cm	0	23	-
PW 4cm	2	23	-
PW 4cm	8	23	-
PW 4cm	26	93	-
PW 4cm	49	182	-
PW 4cm	74	186	-
PW 4cm	81	187	-
PW 4cm	97	201	-
PW 6cm	0	22	-
PW 6cm	8	22	-
PW 6cm	26	22	-
PW 6cm	49	28	-
PW 6cm	74	85	-
PW 6cm	97	151	-

SW=Surface Water, PW=Pore Water, *value is below detection limit of 0.15 ppb MC-LR and is unreliable

Appendix IV: IC Data for Nitrate and Sulfate in Wave Trial

No.	Sample Name	Nitrogen as Nitrate (ppm)	Sulfate ions (ppm)
1	q	n.a.	n.a.
2	q	n.a.	n.a.
3	std b	0.0957	1.0509
4	std 1	0.1518	1.5531
5	std 2	0.2292	2.3017
6	std 3	0.4843	4.8445
7	std 4	0.9492	9.4547
8	std 5	1.9599	19.5741
9	STD 6	5.0256	50.2719
10	STOCK STD	10.2821	n.a.
11	ANION CHECK	0.8388	4.0055
12	USGS 2015 M-216	0.2203	7.6560
13	SW-0 hours	0.1116	1.8871
14	SW- 0 hours	0.1119	2.0717
15	SW- 2 hours	0.1115	2.0108
16	SW- 7 hours	0.1115	2.0235
17	SW- 8 hours	0.1121	2.0238
18	SW- 26 hours	0.1122	2.0141
19	SW- 32 hours	0.1108	2.0401
20	SW- 49 hours	0.1170	2.0913
21	SW- 56 hours	0.1174	2.0886
22	SW- 74 hours	0.1162	2.1025
23	SW- 78 hours	0.1173	2.0325
24	SW- 81 hours	0.1186	2.0771
25	SW- 97 hours	0.1222	2.1171
26	PW 2cm- 0 hours	0.1222	2.1893
27	PW 2cm- 2 hours	0.1588	3.0627
28	PW 2cm- 8 hours	0.1639	3.4882
29	PW 2cm- 26 hours	0.1646	3.9558
30	PW 2cm- 32 hours	0.1764	4.9631
31	PW 2cm- 49 hours	0.1444	3.6163
32	PW 2cm- 56 hours	0.1238	2.1877
33	PW 2cm- 74 hours	0.1645	3.7138
34	PW 2cm- 86 hours	0.1777	3.8236
35	PW 2cm- 97 hours	0.1843	3.7105
36	PW 4cm- 0 hours	0.1837	3.7694
37	PW 4cm- 2 hours	0.2591	7.7913
38	PW 4cm- 8 hours	0.1583	3.8261
39	PW 4cm- 26 hours	0.1442	3.4637
40	PW 4cm- 49 hours	0.1420	3.4046
41	PW 4cm- 74 hours	0.1516	3.6247
42	PW 4cm- 81 hours	0.1566	3.6000
43	PW 4cm- 97 hours	0.1666	3.6178
44	PW 6cm- 0 hours	0.1698	3.7070
45	PW 6cm- 8 hours	0.5171	25.1586
46	PW 6cm- 26 hours	0.2858	10.2944
47	PW 6cm- 49 hours	0.2447	7.7335
48	PW 6cm- 74 hours	0.1696	3.8097

49	PW 6cm- 97 hours	0.1814	3.6988
50	STD 1 .5+4.5	0.0928	1.0173
51	STD 1 .5+4.5	0.0916	n.a.
52	STD 1 .5 +4.5	0.0912	1.0146
53	STD 3 .5+4.5	0.1133	1.2316
54	STD 3 .5+4.5	0.1137	1.2344
55	STD 3 .5+4.5	0.1137	1.2401
56	q	n.a.	n.a.
57	q	n.a.	n.a.
	Sum:	26.855	255.241
	Average:	0.507	5.005
	Rel.Std.Dev:	305.273 %	155.031 %